International Journal of Drug Delivery 5 (2013) 309-322

http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

# Application of Quality by Design Principles to Study the Effect of Coprocessed Materials in the Preparation of Mirtazapine Orodispersible Tablets.

Manal Kamal Darwish[1]

*Corresponding author:

Manal Kamal Darwish

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy; Al-Azhar University, Cairo, Egypt.

## Abstract

The aim of this study was to determine the effect of two coprocessed materials in presence and absence of superdisintegrant (kyron T314) in the preparation of mirtazapine orodispersible tablets. Mirtazapine solubility was increased by complexation with kleptose forming an inclusion complex in a ratio 1:1. Quality by Design (QbD) was incorporated to determine the material attributes and the critical quality attributes (CQAs). Box behnken design was applied to study the effect of three independent variables $X_1$: amount of lufiflash, $X_2$: amount of pearlitol flash and $X_3$: % of kyron T314 on two responses, $Y_1$: dissolution after 1 minute and $Y_2$: disintegration time. All formulated ODTs showed disintegration time less than 35 seconds and all formulations showed a notable increase in dissolution rate. Design space was determined from the overlay plot of different variables, $X_1$ and $X_2$ and $X_3$ at two levels of the superdisintegrant. The one with maximum predicted dissolution rate and minimum predicted disintegration time was a formulation containing ludiflash ($X_1$)= 9.25 mg, pearlitol flash ($X_2$= 50 mg) and kyron T314= 3%. This formulation (Test ODT) was prepared and was subjected to *in vivo* study. Mirtazapine in human plasma was determined by LC-MS/MS and different pharmacokinetic parameters was determined for both test ODT and conventional oral tablet (Remeron). The pharmacokinetic parameters indicated that the two formulations are bioequivalence.

**Keywords:** mirtazapine; Box-Behnken; ludiflash; pearlitol flash; kleptose HPB.

## Introduction

One of the most popular problems that a patient may face during medication is 'dysphagia' or difficulty in swallowing,especially in elderly and pediatrics [1]. To solve this problem, pharmaceutical technologists have devoted considerableefforts for developing a novel type of dosage form fororal administration known as orally disintegrating tablets(ODTs) [2,3].These tablets cause instantaneous disintegration after putting on tongue, thereby releasing the drug quickly when come in contact with the saliva [4].This type of property in dosage form can be attained by addition of different varieties of excipients [5].These excipients need to have better flow, low/no moisture sensitivity, superior compressibility and rapid disintegration ability [6]. One such approach for improving the functionality of excipients is co-processing of two or more excipients.Coprocessing is based on the novel concept of two or more excipients interacting at the sub particle level, the objective of which is to provide a synergy of functionality improvement as well as masking the undesirable properties of individual [7] and lead to formulations with superior properties, likeimproved flow properties, improved compressibility, better dilution potential, fill weight uniformity, and reduced lubricant sensitivity [8].Ludiflash is acoprocessed material consisting of 90% mannitol, 5% Kollidon® CL-SF (crospovidone) and 5% Kollicoat SR 30 D (polyvinyl acetate).Itsone of the novel excipients for fast dissolving drug delivery which disintegrates rapidly within seconds with soft, creamy consistency. Ludiflash decreases the cost because it acts as all-in-one systemlike filler, binder and disintegrant and it givesa faster product development [9]. Pearlitol flash is a new generation of coprocessedmannitol-based excipients for formulation. A combination ofmannitol and starch,both of which are pharmacopoeia-compliant [10,11]. Thus, the effect of such coprocessed materials on the drug release from oral disintegrating tablets even in absence of superdisintegrant was worth studying. The model drug mirtazapine is a noradrenergic and specific serotonergic antidepressant (NaSSA) that acts by antagonizing the adrenergic alpha 2-autoreceptors and alpha2-heteroreceptors as well as by blocking 5-$HT_2$ and 5-$HT_3$ receptors [12]. The aim of this work is to study the effect of the two coprcessed materials, ludiflash and pearlitol flash on the release of mirtazapine from ODTs in presence and absence of superdisintegrant, kyron T314. The study was done by incorporation of a pharmaceutical development process named Quality by Design (QbD). QbD is concerned with the achievement of certain predictable quality with desired and predetermined specifications through relating the critical material attributes and critical process parameters (CPP) to the critical quality attributes (CQAs) of drug product [13, 14].

# Materials and Methods

## Materials

Mirtazapine was obtained from AUG Pharma, Egypt. Ludiflash was obtained from BASF, the chemical company, Germany. Pearlitol flash and kleptose HPB from Roqutte, France.Kyron T314 was obtained from Corel PharmaChem, India. Magnesium stearate from Egyptian International Pharmaceutical Co. EIPICO. Methanol HPLC Grade (Scharlau, spain). Diethyl ether (MTEDA, USA).Ammonium Formate of Reagent Grade (Sigma-Aldrich, Germany).Formic acid of Reagent Grade (Sigma-Aldrich, Germany).n-Hexane 95%(Alliance Bio, U.S.A).

## Methods

### Phase solubility studies for mirtazapine in kleptose HPB.

Phase solubility studies were carried out according to the method reported by Higuchi and Connors. An excess amount of mirtazapine was added to the aqueous solution of kleptose solution (molecular weight =1400) at various concentrations (0.002-0.01M).The contents were stirred for 72 h at37±0.5ºC.After equilibrium, the samples were filtered through 0.45 μm Millipore membrane filtersand absorbance recorded using UV/Vis spectrophotometer (UV-1700 Schimadzu spectrophotometer, Tokyo, Japan) at 292 nm [15].

The apparent stability constant was calculated from the initial straight portion of the phase solubility diagram using the equation:

$$K1:1 = \frac{Slope}{[Intersept(1 - Slope)]}$$

### Preparation of cyclodextrininclusion complexes

### Preparation of cogrindingformulations (CG)

For co-grinding formulations, mirtazapine with kleptose were mixed inthreegeomatrix ratios(1:0.5, 1:1 and 1:2) and triturated in glass mortar pestle for 20 minutes and passed through 80 mesh screen [16].

### Characterization of inclusion complex

### Differential scanning calorimetry (DSC)

Thermal behavior of mirtazapine, kleptoseand inclusion complex were examined using thermalanalyzer(Differential scanning calorimeter, model Mettler DSC60, Switzerland).The sample size was 5 mg and thetemperature range was between 30 and 300 ºC.Nitrogen was used as carrier gas and DSC analysiswas performed at heating rate of 10ºC/min.

### Fourier transfer Infrared spectroscopy (FTIR)

Infrared spectra of pure mirtazapine, kleptose and inclusion compelx were recorded by KBr method usingpyeUnicam SP 1000 IR Spectrophotometer, type pw3710, Holland. Scanning was done from 750 -4000 cm-1.

### In vitro dissolution studies of mirtazapine inclusion complexes

Dissolution of mirtazapine (10 mg) and its inclusion complexes equivalent to 10 mg of mirtazapine was studied using a dissolution apparatus with paddles rotating at 75 rpm.The dissolution was performed in 300 mL of phosphate buffer (pH 6.8) at 37 ± 0.5 C.At fixed time intervals, samples were withdrawn, filtered, and spectrophotometrically assayed for drug content at 292 nm.

### Determination of Quality Target Product Profile (QTPP)

The Target Product Quality Profile (TPQP) is a term that is a natural extension of Target product Profile (TPP) for product quality. It is the quality characteristics that the drug product should possess in order to reproducibly deliver the therapeutic benefit promised in the label. The TPQP guides formulation scientists to establish formulation strategies and keep formulation efforts focused and efficient. The target product intended to be formulated in this work is an orodispersible tablets containing 10 mg mirtazapine. Tablets were formulated to give complete drug release within few minutes. Mirtazapine release was increased by complex formation with kleptose HPB.

### Determination of Material Attributes of excipients and Drug Product CQAs

Material attributes excepients used in the formulation of mirtazapine orodispersible tablets were two coprocessed materials - ludiflash and pearlitol flash- and one super disintegrant (kyron T314). Ludiflash is composed of 90 % mannitol which is a fast dissolving filler with a mildly sweet taste, 5 % kollidon® CL-SF, a superior tablet disintegrant and 5 % kollicoat® SR 30D, a hydrophobic binder for enhanced disintegration. Perlitol flash is a compound of mannitol and starch designed for oral disintegrating tablets. Kyron T-314 is derived from crosslinked polymer of polycarboxylic acids as per USP/NF & has the $K^+$ ionic form. It is a very high purity polymer used in pharmaceutical formulations as a superfast disintegrant as well as dissolution improver in solid dosage forms [17]. CQAs of solid oral dosage forms are typically those aspects affecting product purity, strength, drug release and stability, to achieve this, the percent of drug released after 1 minute and tablet disintegration time were determined to ensure the desired product quality.

### Application of Box Behnkendesign for determiningthe effect of different variables.

Box-Behnken design was applied using three factors and three levels which required 13 experiments.The three factors are $X_1$:amount of ludiflash, $X_2$:amount of pearlitol flash and $X_3$, percentage of superdisintegrant (kyron T314) and are represented by −1, 0 and +1, analogous to the low, middle and high values

respectively. A condition for the Box-Behnken design is that these levels be equally spaced to insure orthogonality (Table 1).
The following equation was built to describe the responses:

$$Y=b_o+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{11}X_{12}+b_{22}X_{22}+b_{33}X_{32}$$

where Y is the response, X the factors and *b* the coefficients of each term calculated by multiple regression analysis. The responses studied were $Y_1$ the percent of drug released after 1 minute and $Y_2$ is the disintegration time of the tablets.

**Table 1.** Experimental domains and coding of the variables

| Variables | Levels | | |
|---|---|---|---|
| | -1 | 0 | +1 |
| Material attributes (process inputs) | | | |
| Amount of ludiflash ($X_1$) | 0 | 25 | 50 |
| Amount of pearlitol flash ($X_2$) | 0 | 25 | 50 |
| Percentage of kyron T314 ($X_3$) | 0 | 3 | 6 |

Responses (CQAs)

$Y_1$ Drug released after 1 minute.
$Y_2$ Disintegration time of the tablets.

## Preparationof mirtazapineorodispersible tablets

Mirtazapine-kleptose complexes were formulated into orodispersible tablets by direct compression method containing drug equivalent to 15mg mirtazapine. All ingredients were properly mixed togetherthen compressed in to tablet by using rotary single punch tablet machine. Different formulations of mirtazapine orodispersible tabletsare shown in Table 2.

**Table 2.** Formulae of orodispersible mirtazapine tablets obtained from Box-Behnken design.

| Ingredients | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ | $F_9$ | $F_{10}$ | $F_{11}$ | $F_{12}$ | $F_{13}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Drug:complex | 94 | 94 | 94 | 94 | 94 | 94 | 94 | 94 | 94 | 94 | 94 | 94 | 94 |
| Ludiflash | 0 | 25 | 50 | 25 | 25 | 25 | 50 | 0 | 50 | 50 | 0 | 25 | 0 |
| Pearlitol | 50 | 25 | 50 | 50 | 50 | 0 | 0 | 25 | 25 | 25 | 25 | 0 | 0 |
| Kyron T314 | 6.6 | 6.6 | 6.6 | 0 | 13.2 | 0 | 6.6 | 0 | 13.2 | 0 | 13.2 | 13.2 | 6.6 |
| Mag. St. | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 |
| Avicel 102 to | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 | 220 |

## Evaluation of mirtazapine ODT

The prepared tablets were evaluated for weight variation, hardnessand % friability. For weight variation, 20 tablets were selected at random and an average weight was determined using Electronic Balance. The hardness of 6 tablets was determined using the Monsanto hardness tester. Friability was determined by first weighing 10 tablets after dusting and placing them in a friability tester (Roche friabilator), which was rotated for 4 min at 25 rpm. After dusting, the total remaining mass of tablet was recorded and the percent friability was calculated [18].

## *In vitro* disintegration time

The disintegration time for all formulations was carried out usingtablet disintegration test apparatus. Six tablets were placedindividually in each tube of disintegration test apparatus and discswere placed. The water was maintained at a temperature of 37 ±2 C and time taken for the entire tablet to disintegrate completelywas noted [18].

## Water absorption ratio

A piece of tissue paper folded twice was placed in a small Petri dish (internal diameter = 6.5cm) containing 5 ml of distilled water. A tablet was placed on the tissue paper. The wetted tablet was weighed. The test was done in triplicate. The water absorption ratio(R) was determined according to the following equation

Water absorption ratio $(R) = \frac{Wa-Wb}{Wb}$X 100

Where, $Wa$ is the weight of the tablet before the test and $Wb$is the weight of the tablet after water absorption [19].

## *In vitro* dissolution study of tablets for ODTs

In-vitro dissolution of mirtazapine ODT was studied using a dissolution apparatus with paddles rotating at 75 rpm. The

dissolution was performed in 300 mL of phosphate buffer (pH 6.8) at 37 ± 0.5 C. At fixed time intervals, samples were withdrawn, filtered, and spectrophotometrically assayed for drug content at 292 nm. Percent drug released after 20 minutes was determined.

## Determination of design space and defining control strategy

The relationship between the process inputs (material attributes X's) and the critical quality attributes (Y's) can be described in the design space [13]. Working within the design space is not considered as a change. Movement outside of the Design space is considered to be a change and would normally initiate a regulatory post approval change.Control strategy is defined as "a planned set of controls, derived from current product and process understanding that assures process performance and product quality". The control strategy in the QbD paradigm is established via risk assessment that takes into account the criticality of the CQA and process capability [20].

## In vivo Study

Six healthy, preferably non-smoking, volunteers, 18-56 years ofage, and within 10% of ideal body weight for height and build, were subjected to single-dose fasting two-ways crossover bioequivalence study with a washout period of seven days.Each volunteer received a single oral dose of test and reference products of mirtazapine as follows; one oral disintegrated tablet of test product (ODT) of 15mg dose and half scored tablet of reference product of 30mg (Remeron®, N.V.Organon, the Netherlands) which is equivalent to 15 mg mirtazapine. The orally disintegrated tablets were orally administered under low light condition during each period of the study under the supervision of a trained Medical Officer. Subjects were instructed to let the tablets completely dissolve on the tongue before swallowing the saliva and then, 240ml of water was administered 30 seconds after drug administration.

The study protocol, which complied with the recommendations of the Helsinki Declaration, was fully approved and performed by Drug Research Centre (DRC, a certified center that performs bioequivalence studies and biowaiver studies based on Central Administration for Pharmaceutical Affairs CAPA guidelines & international regulations on Good Clinical Practice provided by benchmark regulatory bodies like World Health organization (WHO), U.S Food & Drug Administration (FDA), EMEA (European Agency for the Evaluation of Medicinal Products) and ICH (International Conference of Technical Requirements for the Registration of Pharmaceuticals for Human Use).

Blood samples were taken at a frequency sufficient for assessing $C_{max}$, AUC and other parameters. Sampling time of both test and reference tablets was at 0 (pre-dose), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0 and 72.0 hours. Blood samples were collected in heparinized tubes, centrifuged and separated plasma was aspirated and transferred into plastic tubes and were stored at 20 C until assayed.Different parameters were measured such as the area under the plasma/blood concentration-time curve truncated from time (zero) to time (72) ($AUC_{0-72t}$), area under the plasma/blood concentration-time curve from time (zero) to time (infinity) ($AUC_{0-infinity}$), and time to half drug concentration ($t_{½}$). Peak drug concentration ($C_{max}$) and the time to peak drug concentration ($T_{max}$), obtained directly from the data without interpolation.

The assay of mirtazipine in plasma was performed using Liquid chromatography (Agilent 1200 series, USA) coupled with mass spectrometry detection (Agilent 1200 series Triple Quad, USA) operated in positive electrospray ionization mode (ESI). Thermo, Hypersil Gold C8, 4.6 x 50 mm, 5.0 micron analytical column was used and a mobile phase consists of Methanol:AmmFormate pH3.5 (95:5) v/v and a flow rate of 0.6 ml/min.QuetiapineFumaratewas used as an internal standard. Before assay, the linearity, the precision, the accuracy and the selectivity of the method were demonstrated.The Procedure of calculation of mirtazapine in volunteers' human plasma was performed automatically by using Mass Hunter software Program of LC-MS/MS instrument.

# Results and Discusion

## Phase solubility studies

Phase solubility diagram can be classified as ALtype according to Higuchi and Connors as shown in Figure 1. It's clear that the solubility of mirtazapine linearly increased with increasing concentration of kleptose (0.002 M-0.01 M). From the straight line, it was concluded that the increase in solubility was due to formation of 1:1 complex. The solubility constant (Kc) was calculated from the slope of the straight line according to equation,

K1:1 = Slope/Intercept (1 - Slope)

The stability constant was found to be 672.72$M^{-1}$, which is adequate for complex formation.

The phase solubility diagram shows an $A_L$type. The linear mirtazapine-kleptose curve suggested the formation of 1:1 inclusion complex which resulted from molecular interactions between mirtazapine and kleptose.

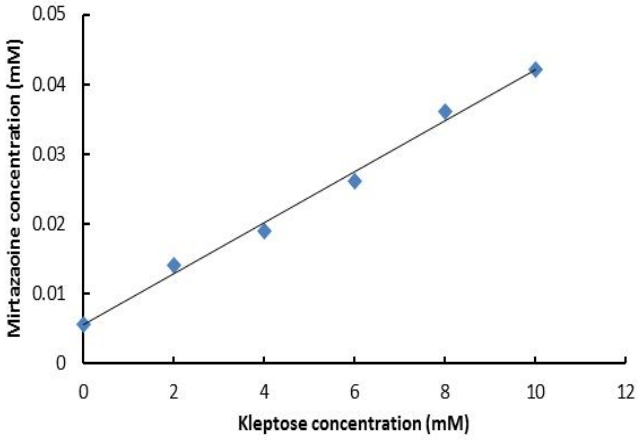



**Figure.1. Phase solubility plot of mirtazapine and kleptose.**

## Characterization of inclusion complex

### Differential scanning calorimetry (DSC)

DSC was carried out to identify the inclusion complex between the drug and kleptose.Figure (2) shows the DSC thermograms for mirtazapine, kleptose and 1:1 inclusion complex. Endothermic peak of mirtazapine appeared at 118 C, which corresponds to its melting point, while kleptose shows a broad endothermic peak at 98.04 C. The intensity of the peak of the drug in the inclusion complex was clearly decreased while that of kleptose was shifted and appeared at 82.67 C. DSC thermogram of mirtazapine-kleptose inclusion complex shows the disappearance or shifting of endothermic peaks of drug. This is mostly an indication of formation of an inclusion complex resulted from entrapment of mirtazapine in the kleptose cavity [21].

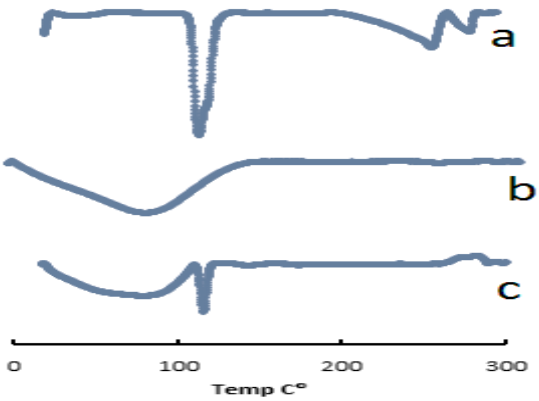



**Figure.2. DSC thermogram of (a) mirtazapine, (b) kleptose and (c) inclusion complex**

### Fourier transfer Infrared spectroscopy (FTIR)

IR spectroscopy of mirtazapine (Figure 3a) shows C-H stretching vibrations band of methyl group at 2931 $cm^{-1}$. Methyl group attached to a $N_2$ atom gives rise to a band at 2854 $cm^{-1}$ (22). Bands for C-C stretching of the phenyl group appeared at 1585 $cm^{-1}$ and 1444 $cm^{-1}$. The primary aromatic amines with N directly on the ring give bands at 1336-1200 $cm^{-1}$. The benzene ring C-H appears in the range of 1359-1074 $cm^{-1}$ and 788-636 $cm^{-1}$ for the in plane and out of plane bending vibrations respectively (23&24). The IR spectroscopy of kleptose (Fig. 3b) is characterized by large peaks at 3408 $cm^{-1}$ characterized for O-H, 2926 $cm^{-1}$ for C-H, 1647 $cm^{-1}$ for H-O-H bending and 1029 $cm^{-1}$ for C-O-C. Figure 3c shows the IR spectrum for the inclusion complex. The broad peak for O-H group appeared in the inclusion complex. All drug peaks were smoothed and the peak at 1647 $cm^{-1}$ in kleptose was disappeared. IR spectroscopy for the inclusion complex shows a strong physical interaction between the pure mirtazapine and kleptose and was illustrated by the smoothness of all drug peaks. Another proof for the interaction was seen by the disappearance of the peak at 1647 $cm^{-1}$ in kleptose (for water of crystallization) which means that mirtazapine replaces water in the cyclodextrin cavity of kleptose. The overall interaction is said to be noncovalent as no new peaks were seen in the inclusion complex [25]. This could be explained on the basis of the ability of cyclodextrin- drug complex to modify the physicochemical properties of drugs such as crystal habit and solubility and thereby forming a highly water soluble amorphous forms [26]. Kleptose which is hydroxypropylbetacyclodextrins are purified polydisperse products resulting from the controlled reaction of propylene oxide and native betacyclodextrin [27].

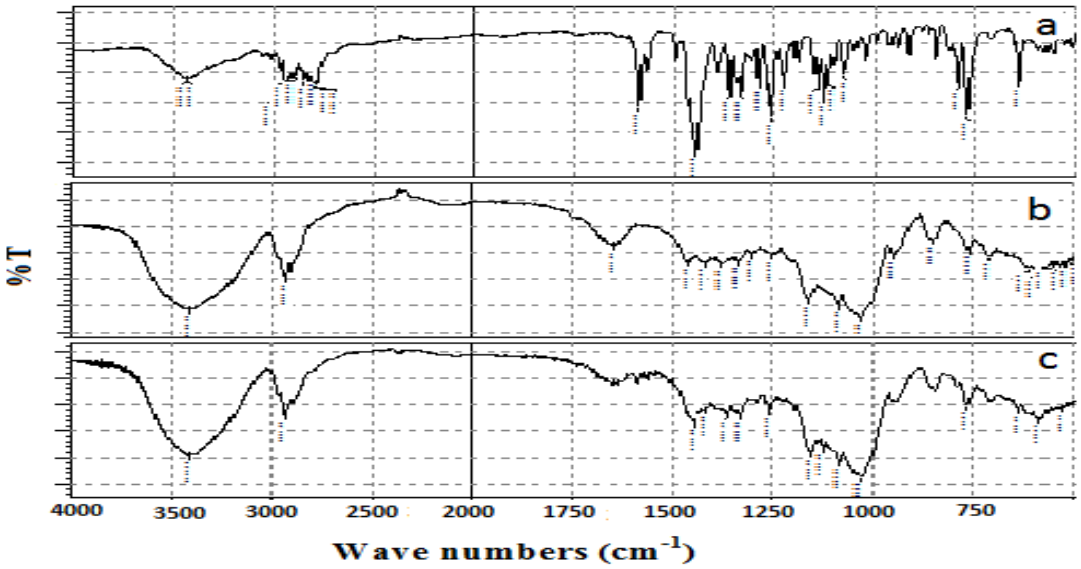



**Figure.3**. FT-IR spectra of a: pure mirtazapine, b: kleptose HPB and c: inclusion complex

## In-vitro dissolution of mirtazapine inclusion complex

Figure 4 shows the dissolution profile of pure mirtazapine and it's inclusion complexes. It was found that only 19.21% of pure mirtazapine was released after 20 minutes while a complete drug release was obtained from the inclusion complex (ratio 1:1) after the same period. Figure (4) also shows that mirtazapine inclusion complex ratio 1:1 gave highest drug release in comparison with the other two ratios 1:0.5 and 1:2.

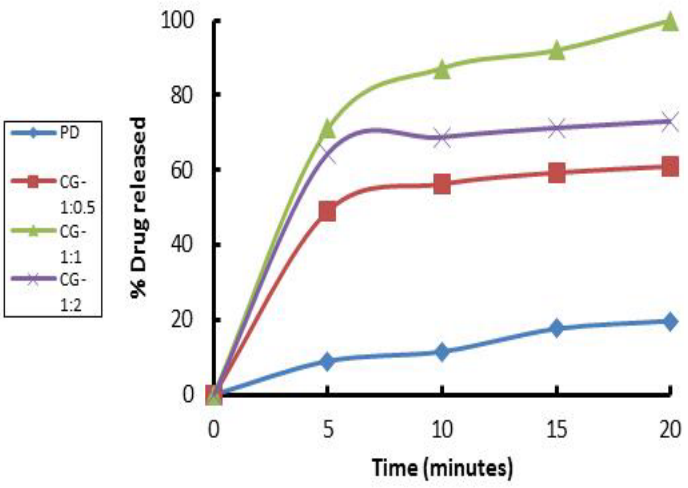



**Figure.4. % Drug released from pure mirtazapine (PD) and from coground (CG) inclusion complexes in different ratios.**

## Evaluation of mirtazapine ODTs

The drug content was found to be from 98.10±0.51 to 101.01±0.43as shown in Table 3. The results were within the range that indicated uniformity of mixing of the drug with excipients in the developed formulations. Friability of all formulation was in the range of from 0.58±0.05 to 0.89±0.02 % which was found to be within the approved range (<1%) in all formulations. The hardness of the tablets was found in the range of 3.01±0.30 to 4.61±0.62 kg/cm2. The average percentage weight variation was found within the pharmacopoeial limits of ±10%. The obtained results were found to be from 218.3±1.52 to 221.9±2.08.

## In vitro disintegration time

The disintegration time recorded for all the formulation was found in the range of 10±0.60 to 33±0.70 seconds. All the formulations were disintegrated in less than 35 seconds. The results are shown in Table 3.

## Water absorption ratio

Formulation containing both pearlitol flash and ludiflash in addition to kyron T314 showed highest water absorption ratio ( F3: 85.3±0.51 %). Minimum water absorption ratio was seen in formulation $F_{13}$ which deprived from both pearlitol flash and ludiflash (Table 3).

**Table 3**. Evaluation of different mirtazapine formulations

| Parameter | Uniformity of Content (%)±SD | Friability (%) ±SD | Hardness (kg/cm$^2$) ±SD | Weight variation (mg)±SD | Water absorption Ratio (%) ±SD | % Drug released (1 min)* ($Y_1$)±SD | Disintegration time($Y_2$) (seconds) ±SD |
|---|---|---|---|---|---|---|---|
| $F_1$ | 99.45±0 .43 | 0.73±0.03 | 3.49±0.36 | 219.4±1.59 | 74.0±0.52 | 62.5±0.62 | 28±0.57 |
| $F_2$ | 98.54±0.50 | 0.77±0.03 | 3.88±0.49 | 221.9±2.08 | 67.1±1.0 | 59.9±0.51 | 12±0.76 |
| $F_3$ | 100.2±0.50 | 0.89±0.02 | 3.29±0.31 | 220.1±1.56 | 85.3±0.51 | 66.1±0.72 | 10±0.60 |
| $F_4$ | 99.77±0.57 | 0.83±0.03 | 4.61±0.62 | 220.9±1.59 | 81.5±0.76 | 63.3±0.66 | 16±0.76 |
| $F_5$ | 98.10±0.51 | 0.69±0.02 | 3.96±0.33 | 218.3±1.52 | 82.4±0.45 | 56.4±0.51 | 22±1.50 |
| $F_6$ | 101.01±0.43 | 0.87±0.01 | 4.11±0.51 | 220.4±2.07 | 51.9±0.70 | 48.6±0.82 | 31±0.61 |
| $F_7$ | 100.91±0.50 | 0.81±0.04 | 4.15±0.61 | 221.8±1.91 | 58.5±0.90 | 53.8±0.42 | 29±0.72 |
| $F_8$ | 99.19±0.49 | 0.87±0.03 | 3.93±0.41 | 219.8±2.04 | 63.4±0.51 | 49.9±0.70 | 31±0.51 |
| $F_9$ | 100.73±0.43 | 0.85±0.04 | 3.25±0.56 | 221.5±2.01 | 77.9±0.55 | 48.2±0.66 | 20±0.80 |
| $F_{10}$ | 98.27±0.50 | 0.78±0.03 | 3.08±0.44 | 219.0±1.82 | 76.8±0.52 | 49.1±0.64 | 25±0.50 |
| $F_{11}$ | 99.74±0.50 | 0.72±0.03 | 4.09±0.38 | 221.8±1.62 | 70.8±0.51 | 39.5±0.72 | 33±0.70 |
| $F_{12}$ | 99.31±0.43 | 0.58±0.05 | 3.12±0.53 | 221.4±1.90 | 53.4±0.50 | 43.3±0.91 | 28±0.73 |
| $F_{13}$ | 100.08±0.50 | 0.77±0.04 | 3.01±0.30 | 220.0±1.72 | 49.3±0.41 | 48.2±0.55 | 31±0.65 |

* Percent drug released after 1 minute.

## *In vitro* dissolution study of mirtazapine ODTs

Figure 5 shows the percent drug released from different formulations. It is clear that the presence of the two coprocessed material resulted in a notable increase in mirtazapine release even in absence of the superdisintegrant. Formulation $F_2$ gave complete drug release within 15 minutes. Presence of kyron T314 in concentration 3% gave higher drug release than 6%.

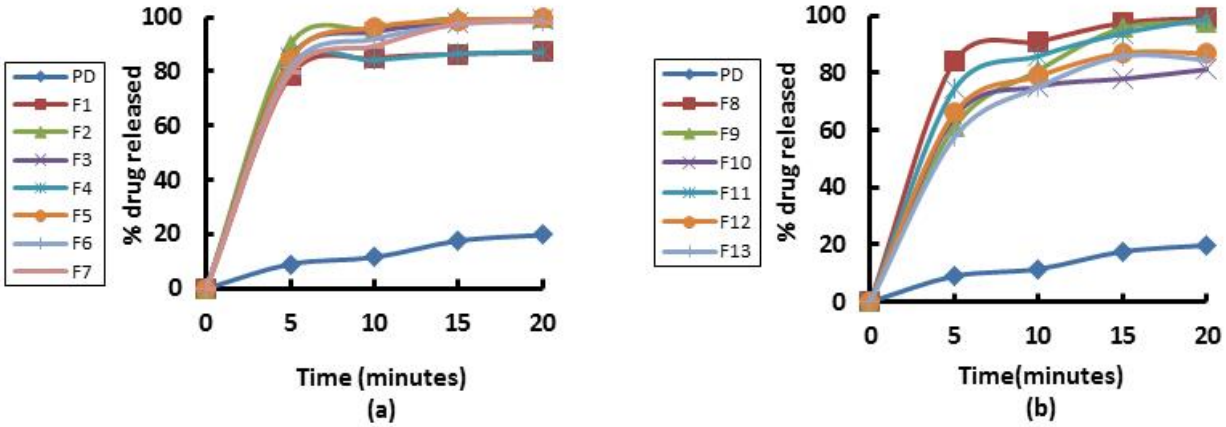



**Figure.5.** % Drug released from different mirtazapine formulations, a: formulations from 1-7 and b: formulations from 8-13.

In vitro drug release from inclusion complex shows a complete mirtazapine release within 20 minutes due to high water solubility of kleptose. Mirtazapine orodispersible tablets containing pearlitol flash showed high water absorption ratio due to starch hydrophilicity that gives good wettability properties. In case of formulations with ludiflash, the high retention capacity of water was due to kollidon® CL-SF with very small particles and high absorption rate. Incorporation of two coprocessed materials led to a notable increase in water absorption rate which was reflected on a rapid disintegration time. Kyron T314 breaks the tablets into very smaller particles, thus it increases the effective surface area for the absorption of the active substances and thus it decreases the disintegration time and then the dissolution [28].

## Experimental design

Box Behnken design was applied and the material attributes were determined to be amount of ludiflash ($X_1$), amount of pearlitol ($X_2$) and percentage of kyron ($X_3$). The CQAs were drug released after minute ($Y_1$) and disintegration time of the tablets ($Y_2$). The material attributes and response variables were studied and related to determine the effect of each factor on the determined responces using Design Expert-8 [29].

Results shows that the percent drug released after one minute ($Y_1$) ranged from 39.5% in $F_2$ to 66% in $F_7$. The polynomial equation obtained for this response was:

$Y_1$ (dissolution after 1 minute) = 60.00 + 2.18 A + 6.83 B - 2.88 C – 0.45 AB + 2.35 AC - 0.40 BC - 4.28 $A^2$ + 1.72 $B^2$ – 9.12 $C^2$.

The range of responses for $Y_2$ was found to be 10 seconds in $F_3$ to 33 seconds in $F_6$. The polynomial equation obtained for this response was:

$Y_2$ (disintegration time) = 11.88 – 4.88 A – 5.37 B + C – 4 AB – 1.74 AC + 2.25 BC +7.81 $A^2$ + 4.81 $B^2$ + 7.56 $C^2$.

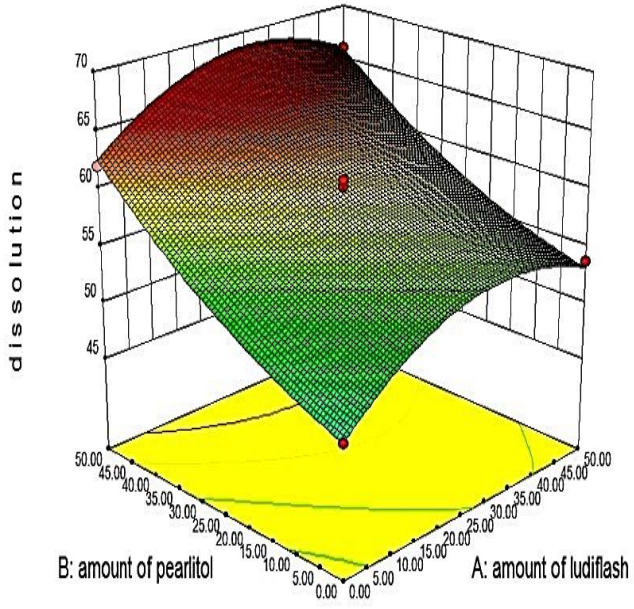
The equations represent the quantitative effect of process variables ($X_1$, $X_2$, and $X_3$) and their interactions on the responses ($Y_1$ and $Y_2$). The values of $X_1$, $X_2$, and $X_3$ were substituted in the equation to obtain the theoretical values of $Y_1$ and $Y_2$.

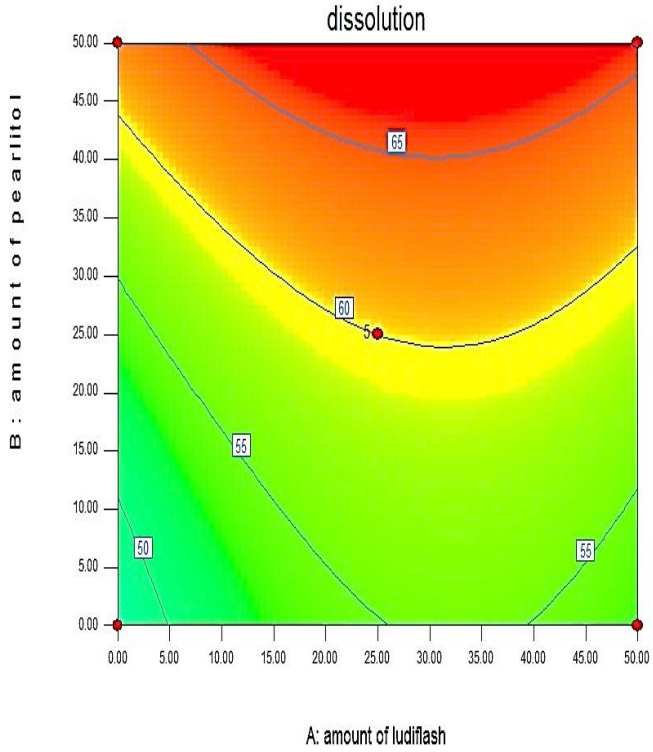
Based on the experimental design and factor combination, a quadratic model was found to be significant for percent drug released after 1 minute ($X_1$)with *F* value of 235.76 and *P* value < 0.0001 and for tablet disintegration time ($X_2$) with *F* value of 155.50 and *P* value < 0.0001.

Table (4) shows the analysis of variance for the first response ($Y_1$: percent drug released after 1 minute). This model shows a nonsignificant lack of fit of value = 0.84. It also shows the analysis of variance for the second response ($Y_2$: disintegration time). This model shows a nonsignificant lack of fit of F value = 5.72. It can be concluded that all factors A (amount of ludiflash), B (amount of pearlitol) and C (percent of disintegrant) significantly affected the two responces $Y_1$ and $Y_2$(Table 4).

**Table 4**. Analysis of variance for $Y_1$ (dissolution after 1 minute) and for $Y_2$ (disintegration time).

| Source | Sum of Squares | Mean Square | F value | p-value Prob>F |
|---|---|---|---|---|
| **Model ($Y_1$)** | 950.29 | 105.59 | 235.76 | < 0.0001 |
| A | 37.85 | 37.85 | 84.50 | < 0.0001 |
| B | 372.65 | 372.65 | 832.06 | < 0.0001 |
| C | 66.13 | 66.13 | 147.65 | < 0.0001 |
| AC | 0.81 | 0.81 | 1.81 | 0.2206 |
| BC | 22.09 | 22.09 | 49.32 | 0.0002 |
| AB | 0.64 | 0.64 | 1.43 | 0.2708 |
| $A^2$ | 76.95 | 76.95 | 171.82 | < 0.0001 |
| $B^2$ | 12.53 | 12.53 | 27.98 | 0.0011 |
| $C^2$ | 350.59 | 350.59 | 782.82 | < 0.0001 |
| **Model ($Y_2$)** | 1424.73 | 158.30 | 216.94 | < 0.0001 |
| A | 144.50 | 44.50 | 198.02 | < 0.0001 |
| B | 364.50 | 364.50 | 499.51 | < 0.0001 |
| C | 4.50 | 4.50 | 6.17 | 0.0420 |
| AC | 90.25 | 90.25 | 123.68 | < 0.0001 |
| BC | 20.25 | 20.25 | 27.75 | 0.0012 |
| AB | 42.25 | 42.25 | 57.90 | 0.0001 |
| $A^2$ | 232.75 | 232.75 | 318.97 | < 0.0001 |
| $B^2$ | 148.31 | 148.31 | 203.25 | < 0.0001 |
| $C^2$ | 299.58 | 299.58 | 410.54 | < 0.0001 |



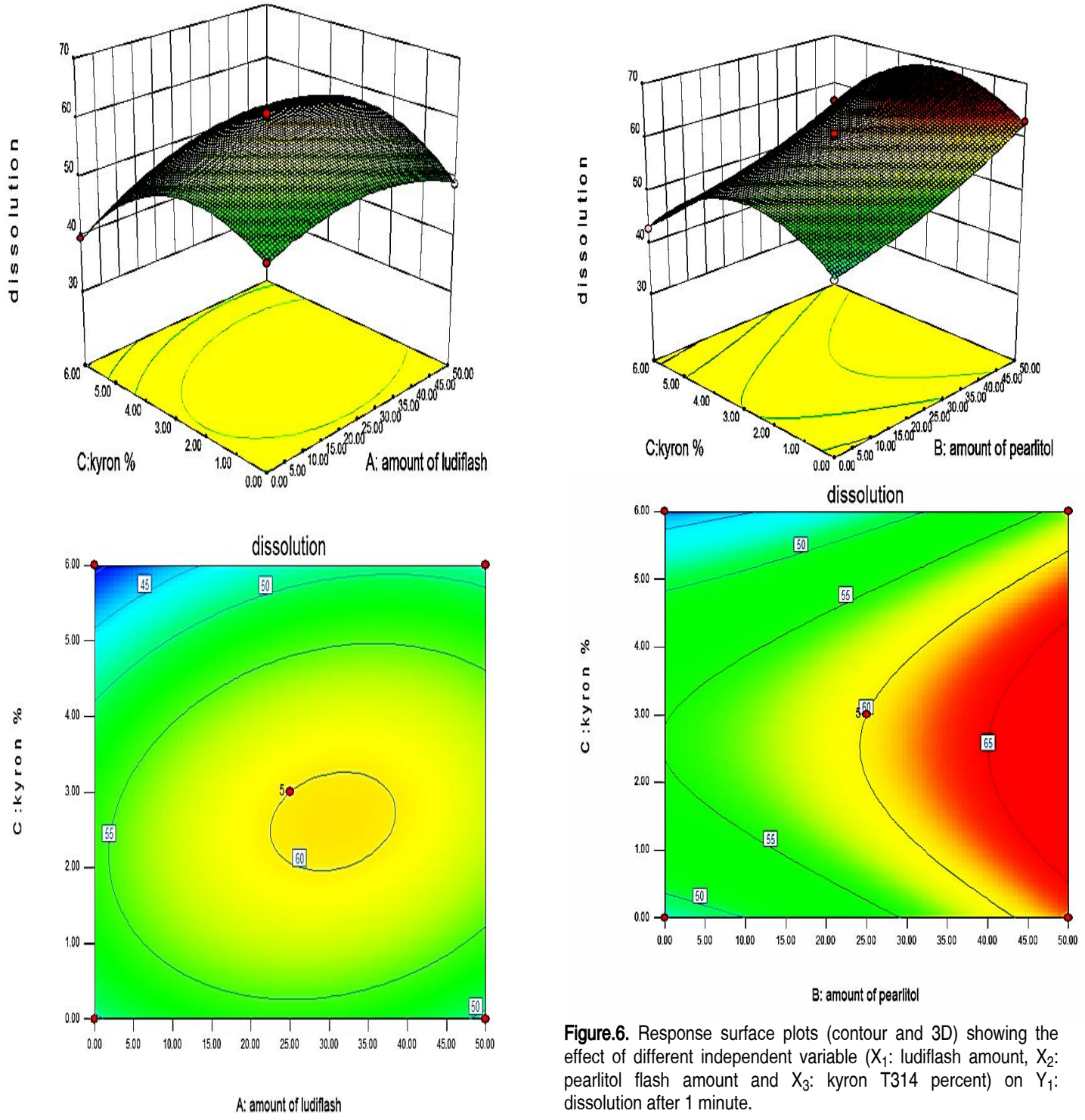
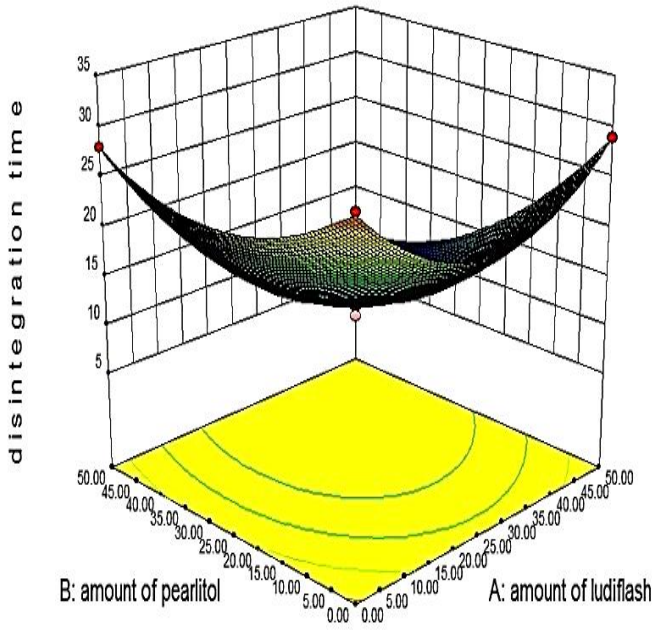
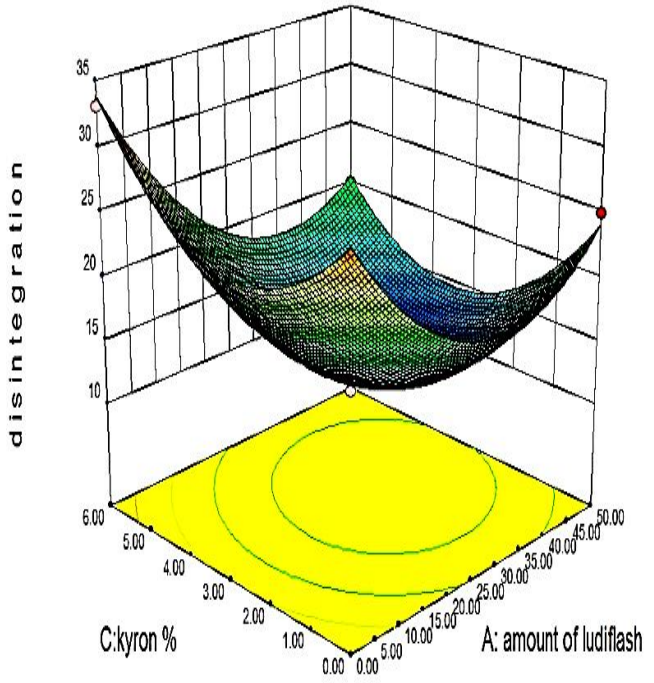
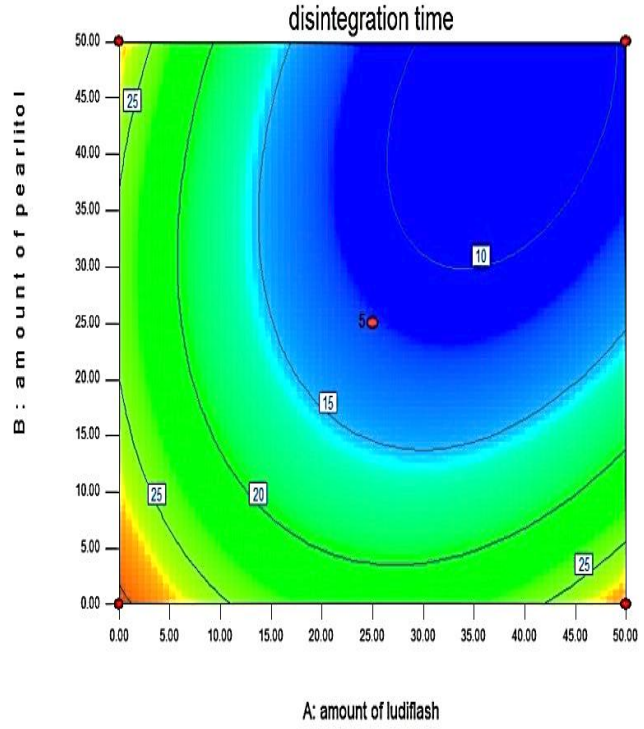
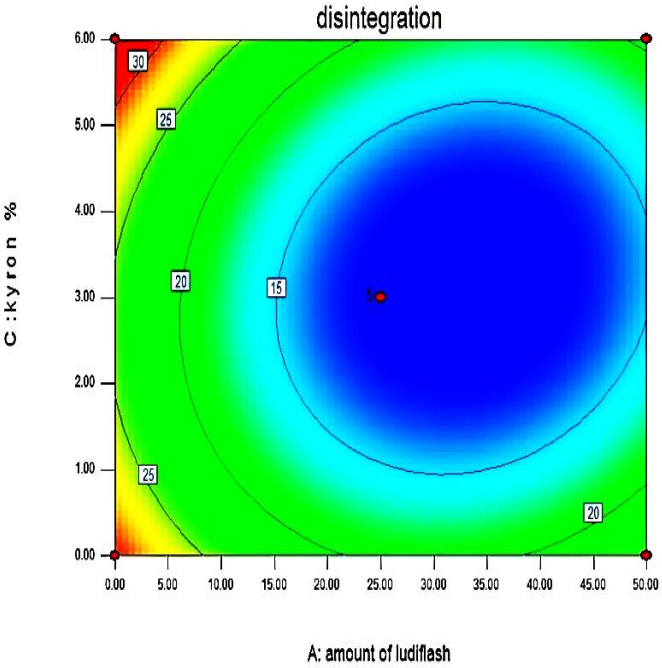



**Figure.6.** Response surface plots (contour and 3D) showing the effect of different independent variable ($X_1$: ludiflash amount, $X_2$: pearlitol flash amount and $X_3$: kyron T314 percent) on $Y_1$: dissolution after 1 minute.


disintegration time
B: amount of pearlitol
A: amount of ludiflash


disintegration
C:kyron %
A: amount of ludiflash


disintegration time
B: amount of pearlitol
A: amount of ludiflash


disintegration
C: kyron %
A: amount of ludiflash

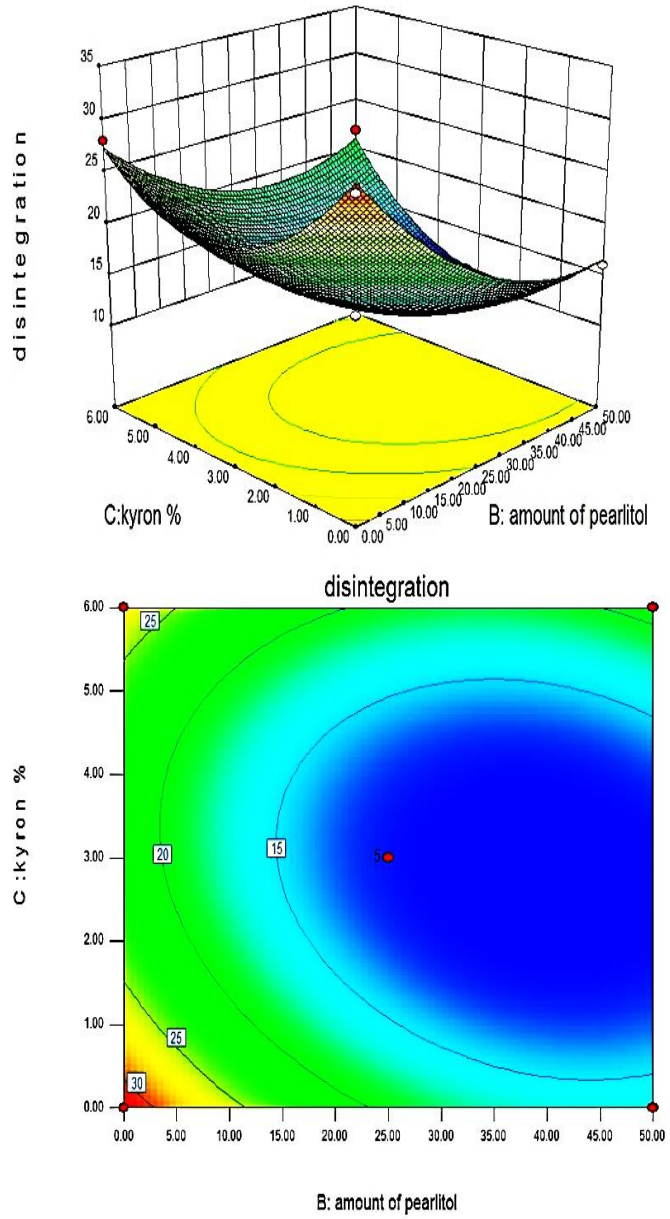
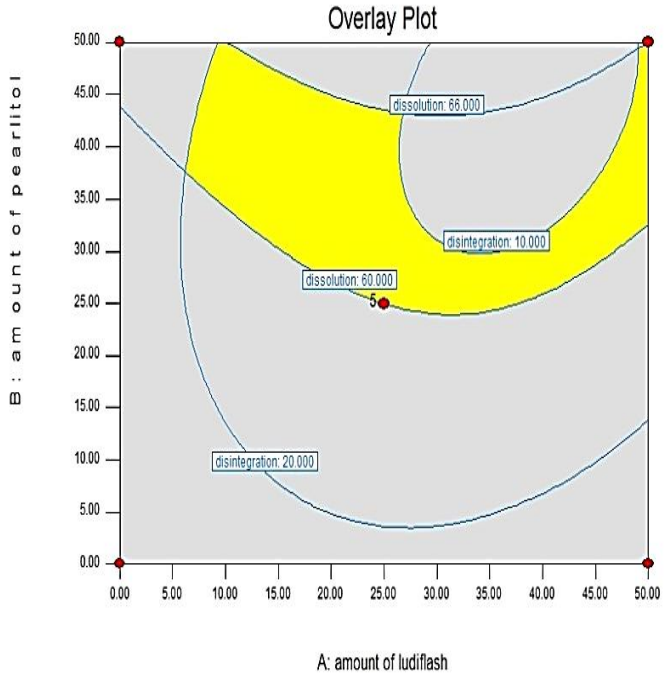



**Figure.7**. Response surface plots (contour and 3D) showing the effect of different independent variable ($X_1$: ludiflash amount, $X_2$: pearlitol flash amount and $X_3$: kyron T314 percent) on $Y_2$: disintegration time.

The relationship between the dependent and independent variables were further elucidated using contour plots and response surface plots. Figure (6) shows the response plots (3D) and the contour plots for the effect of factors $X_1$, $X_2$, and $X_3$ on the first response $Y_1$. It was observed that increasing the amout of ludiflash and pearlitol flash increases the dissoltion, being more clear in case of pearlitol flash. Increasing the percent of kyron till 3% increases the dissolution to a certain limit, after which dissolution started to decrease. Regarding the second response (disintegration time), increasing the amount of all factors decreases the disintegration time and after reaching certain values, increasing thses factors, lead to an increase in the disintegration time as shown in Figure (7).

## Determination of design space and control strategy

With multiple responses one need to find regions where requirements simultaneously meet the critical properties (the *sweet spot)*. By superimposing or overlaying critical response contours on a contour plot, one can visually search for the best compromise. In other words, design space can be determined from the common region of successful operating ranges for multiple CQAs. Figure (8) shows the determined design space composing of the overlapping region of different ranges of the CQAs in two conditions, kyron T314 (3%) and in absence of superdisintegrant (zero %). In the first condition as seen in the Figure, the two optimum values for ludiflash and pearlitol flash were given and they were ($X_1$=9.25 mg, $X_2$=50 mg) and ($X_1$=36.67 mg, $X_2$=5.36 mg). For the second condition, the absence of superdisintegrant gave rise to two optimum values and they were, ($X_1$=16 mg, $X_2$=50 mg) and ($X_1$=50 mg, $X_2$=50 mg). Among all mentioned values, the one with maximum predicted dissolution rate and minimum predicted disintegration time was a formulation containing ludiflash ($X_1$)= 9.25 mg, pearlitol flash ($X_2$= 50 mg) and kyron T314= 3%. This formulation (Test ODT) was prepared and was subjected to *in vivo* study.



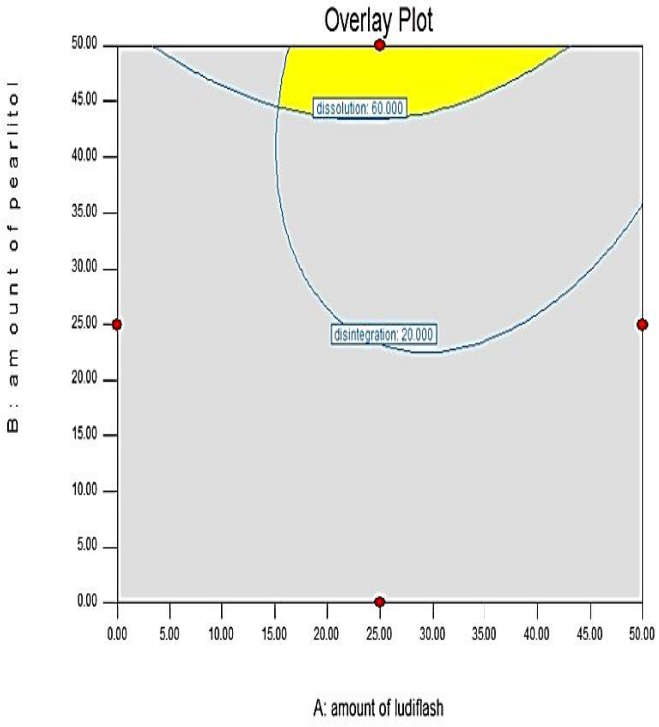



**Figure. 8.** Overlay plots showings the effect of different variable ($X_1$ and $X_2$) on the dependent variable: ($Y_1$ and $Y_2$) at zero %superdisintegrant (right) and at 3% super disintegrant (left).

## Validation of procedures for the determination of mirtazapine in Human Plasma by LC-MS/MS.

Mirtazapine was subjected to analytical validation in human plasma using an LC-MS/MS method and the procedure of calculation of mirtazapine in volunteers' human plasma was performed automatically by using Mass Hunter software Program of LC-MS/MS instrument.

## Calibration curve validation.

Calibration curve validation was developed and the regression equation was found to be Y= 0.027498x + 0.032262, with the determination coefficient of 0.999168. The calibration curve shows linearity over the range from 2.5- 150 ng/ml.

## Precision and accuracy

Precision and accuracy was assessed at within-day bases (intra-batch), which defines those parameters during a single analytical run; and at between-day basis (inter-batch), which measures the between day variability. Inter- and intraday accuracy shows an average recovery % of 99.75 and 99.34% respectively. The lowest standard value(2.5ng/ml) proved to be accepted as the lower limit of quantitation (LOQ) of the method. All parameters were in accordance to the FDA Guidelines (less than 15% for precision and inside 80-120% for the accuracy.

## Selectivity

The selectivity was proved by the injection of pooled blank human plasma from different sources and no interference was observed in chromatograms. There were no interfering peaks at the retention of mirtazapine or the internal standard.

## Pharmacokinetic Parameters

The mean plasma concentration *vs*. time profiles for both ODT and conventional oral tablet are shown in Figure 9. The mean pharmacokinetic parameters calculated from individual plasma mirtazapine concentrations *vs*. time profiles are summarized in Table 5. It was found that the 90% confidence interval limit (range) of $C_{max}$, $AUC_{0-72}$ and $AUC_{0-\infty}$ for mirtazapine was found to be 100.08, 99.86 and 99.85% respectively, which were within the accepted range of FDA (80-120%). Different pharmacokinetic parameters suggested that two products are bioequivalence.

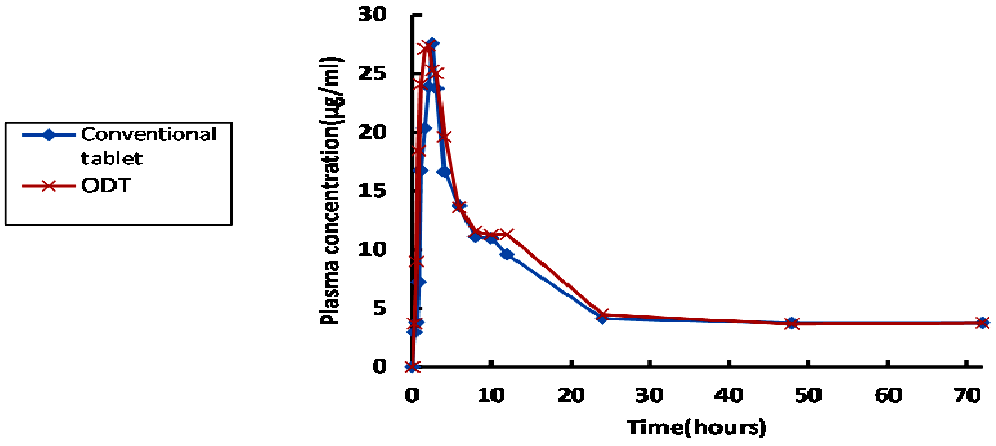



**Figure.9. Mean plasma concentration-time profile of mirtazapine in ODT in comparison to conventional tablets**

**Table 5**. Mean pharmacokinetic parameters for mirtazapinein ODT and conventional oral tablets administered to six healthy volunteers

| Pharmacokinetic Parameters. | ODT | Conventional oral tablet |
|---|---|---|
| AUC $_{0\text{-}72}$(μh/ml) | 480.03 | 436.949 |
| AUC $_{0\text{-}}$ (μh/ml) | 683.94 | 650.548 |
| $t_{1/2}$ (h) | 37.37 | 40.2755 |
| $T_{max}$ (h) | 1.83 | 2.58333 |
| $C_{max}$ (μg/ml) | 34.35 | 31.622 |

## Conclusion

In this study, mirtazapine solubility was increased by complexation with kleptose HPB, then orodispersible tablets were prepared using two different coprocessed materials, ludiflash and pearlitol flash. Box-Behnken design was used to investigate the influence of different formulation variables on the prepared tablets.Mirtazapine in human plasma was determined by LC-MS/MS and different pharmacokinetic parameters was determined for both test ODT and conventional oral tablet (Romeron). The pharmacokinetic parameters indicated that the two formulations are bioequivalence.

## References


[1]. Kaur T, Bhawandeep G, Sandeep K, Gupta G D. 2011. Mouth dissolving tablets: a novel approach to drug delivery. Int J Curr Pharm Res. 3:1-7.

[2]. Indhumathi D, Rathnam G. 2010. Design and optimization of orodissolving tablet of antidepressant drug by superdisintegrants addition method. Int J Pharm Sci Rev Res. 2(2):1-8.

[3]. Bhardwaj V, Bansal M, Sharma PK. 2010. Formulation and Evaluation of Fast Dissolving tablets of amlodipine besylate using different super disintegrants and camphor as sublimating agent. AmEur J Sci Res. 5(4):264-269.

[4]. Wagh, MP, Yewale CP, Zate SU, KothawadePI, Mahale GH. 2010. Formulation and evaluation of fast dispersible tablets of aceclofenac using different superdisintegrants. Int J of Pharm Sci. 2:154-157.

[5]. Sunita AC, Ankit BC, Tejal AM. 2010. Excipients Updates for Orally Disintegrating Dosage Forms. Int J Res Pharm Sci.1(2):103-107.

[6]. Avachat A, Ahire VJ. 2007. Characterization and evaluation of spray dried co-processed excipients and their application in solid dosage forms. Indian J Pharm Sci. 69(1):85-90.

[7]. Nagendrakumar D, Raju SA, Shirsand SB, Para MS. 2010. Design of fast dissolving granisetronHCL tablets using novel co-processed superdisintegrants. Int J PharmaSci Rev Res.1(1):58-62.

[8]. Nachaegari SK, Bansal AK. 2004. Co-processed excipients for solid dosage forms. Pharm Technol. 28(1):52-64.

[9]. Sandra K, Silke G, Karl k. 2007. Ludiflash® – Easy and reliable development of orally dispersible tablets. 2004. Excipients & Actives for Pharma.19:2-4.

[10]. Joshi AA, Duriez X, Added Functionality Excipients: An Answer to Challenging Formulations. Pharm. Technol. Suppl.19:12–19.

[11]. Bansal AK. 2003. Improved excipients by solid-state manipulation. The Ind Pharmacist. 31:9-12.

[12]. Anttila SA, Leinonen EV.2001. A review of the pharmacological and clinical profile of mirtazapine.CNS Drug Rev.7:(3):249-64.

[13]. Food and Drug Administration. Guidance for industry ICH Q8(R2) Pharmaceutical Development. (Step 4, 2006).

[14]. Emad B, Wessam E, Omaima E.2011. Application of pharmaceuticalQbD for enhancement of the solubility and dissolution of a class II BCS drug using polymeric surfactants and crystallization inhibitors: development of controlled-release tablets. AAPS PharmSciTech. 12(3):799-810.

[15]. Higuchi T, Connors KA. 1965. Phase solubility techniques. Adv Anal Chem Instr. 4:117-212.

[16]. Friedrich H, Nada A, Bodmier R. 2005. Solid state and dissolution rate characterization of co-ground mixtures of nifedipine and hydrophilic carriers. Drug DevInd Pharm. 31(8):719–728.

[17]. Swati J, Monali S, Ajay B, Janardan L, Bhanudas K, Anuruddha C. 2010. Development of palastile release tablets of atenolol with swelling and pulsatile layers. Int J Appl Pharm. 2(3):31-40.

[18]. Patel B, Patel D, Parmar R, Patel C, Serasiya T, Sanja SD. 2009. Development and in vitro evaluation of fast dissolving tablets of glipizide. Int J Pharm Pharm Sci. 1:145–50.

[19]. Chaudhari PD, Chaudhari SP, Lanke SD. 2007. Formulation and in vitro evaluation of taste masked orodispersible dosage form of levocetrizinedihydrochloride. Indian J Pharm Educ Res. 41:319-28.

[20]. Amit SP, Anil MP. 2013. Quality by Design (QbD): A new concept for development of quality pharmaceuticals. Int J Pharm Qual Ass. 4(2):13-19.

[21]. Vivek K, Reddy H and Murthy RR. 2007. Investigation of the effect of the solid lipid matrix on drug entrapment, in-vitro release, physical stability of olanzapine-loaded solid lipid nanoparticles. AAPS Pharm. Sci. Tech. 8: 1-17.

[22]. Yavuz AE, Haman SB, N. Kazanc N. 2009. Structural and vibrational study of maprotiline. J MolStruc. 924/926:313-321.




[23]. Colthup NB, Daly LH, Wiberley SE. 1990. Basic theory. In: Introduction to the infrared and Raman spectroscopy. John RF, Kazuo N (eds). Third ed. Academic press: Boston. pp. 1-89.

[24]. Seda GS, Ayse ES. 2013. Molecular structure, FTIR, FT-Raman, NMR studies and first order molecular hyperpolarizabilities by the DFT method of mirtazapine and its comparison with mianserin. SpectrochimicaActaMolBiomolSpectros. 104(1):222-234.

[25]. George SJ, Vasudevan DT. 2012. Studies on the preparation, characterization, and solubility of 2-HP-β-cyclodextrin-meclizine HCl inclusion complexes. Pharmaceutics. 4(4):220-227.

[26]. Patil JS, Kadam DV, Marapur SC, Kamalapur MV. 2010. Inclusion complex system; A novel technique to improve the solubility and bioavailability of poorly soluble drugs: A review. Int J Pharm Sci Rev Res. 2(2):29-34.

[27]. Marcus EB, Thorsteinn L. 2007. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Deliv Rev. 59:645–666.

[28]. Gandhi BR, Mundada AS, Gandhi KR. 2011. Evaluation of Kyron T-314 (Polacrillin Potassium) as a novel super disintegrant. Int J Drug Deliv.3:109-114.

[29]. Design Expert. Version 8.0.7.1, State-Ease Inc., Minneapolis, MN, USA.